Metal-ion sites as structural and functional probes of helix-helix interactions in 7TM receptors.

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Abstract.
Mutagenesis of the tachykinin NK-1 receptor(1) and κ-opioid receptor(2) have shown that metal-ion sites can be engineered in the membrane embedded helices of 7 transmembrane G-protein coupled receptor (7TM) receptors.The metal-ion sites can compete for the receptor both with radiolabelled agonist and antagonist(2), and can efficiently antagonize the receptor(1). The engineering of metal-ion sites imposes a constraint on the possible distance, a "NOE", between the chelating ligands in models of the NK-1 and κ-opioid receptors. Here, we have tested the helical connectivity and rotation of a 7TM receptor, by engineering bis-histidine zinc-sites in the transmembrane helices of the NK-1 receptor. The original zinc-site, between transmembrane V and -VI(1) was redesigned to involve residues on transmembrane III. Using this information a new site was designed involving residues on transmembrane II and -III. The functional properties of the constructs were tested. We propose that the transmembrane helices are oriented in an anti-clockwise manner (looking from the extracellular side). It is suggested that Zn(II) act as an "allosteric antagonist" by stabilizing inactive conformations of the mutant receptors.

References

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